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RESEARCH ARTICLE

Effect of temperature and relative humidity on seed viability and storage of senna (*Cassia angustifolia* Vahl.) seeds

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SUMMARY

The seed material was stored at four different levels of relative humidity (75 %, 33%,11% and 5.5%) and three different storage temperatures (5°C, 20°C and ambient) using saturated salt solutions in airtight desiccators. Observations on various physiological and biochemical parameters were recorded on monthly intervals. Storage temperatures of 5°C and 20°C at 5.5 per cent and 11 per cent relative humidity were found to be optimal, extending seed storability in senna. Seeds stored at controlled condition and the seeds that were kept under low relative humidity (5.5%and 11%) showed no change in all the physiological parameters like seed germination (%), vigour index, root length shoot length and speed of germination. The biochemical parameters like electrical conductivity and lipid peroxidation showed significant increase in value with loss of viability under different treatments with increase in storage time. Total soluble proteins and the activity of enzymes like dehydrogenase and acid phosphatase were positively correlated with seed viability and the amount decreased with storage period. The amount of total soluble sugars progressively increased with storage period in all the treatments. Senna seeds are tolerant to ultra desiccation and this technique can be successfully used for cost effective conservation of this species

Key Words **:** Seed ageing, Germination, Seed vigour, Protein profile, Lipid per oxidation

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Senna (*Cassia angustifolia* Vahl.), belonging to sub family Caesalpinaceae (family Leguminosae), is locally known as Sonamukhi and Sanay in Hindi and as Tinnelveli Senna in Tamil Nadu. It is a legume plant native of Saudi Arabia. It is widely used in ayurveda and unani system of medicine mainly because of its laxative property of its aerial parts. The laxative property is mainly due to chemicals namely sennosides A, B, C and D. Although the pods contain higher percentage of total sennosides (3-5%) than the foliage (2-4%), the demand for leaves is high for use in ayurvedic preparations, herbal tea, bakery products and other home preparation. It is grown as an annual crop of 5-7 months duration in approximately 10,000 ha both as irrigated as well as rainfed crop in India (Gupta and Pareek, 1995). The major part of the produce is exported as leaves and sennoside concentrates.

A little study has been carried out on the seed viability and conservation aspects, hence, the present investigation was undertaken to study the effect of various temperature and relative humidity conditions on different physiological and biochemical parameters of seed for effective conservation.

MATERIALS AND METHODS

The freshly harvested seeds of senna (*Cassia angustigolia*) were collected from Virudhunagar district of Tamil Nadu in the month of September-October 2008.One set of 500 seeds was left at ambient laboratory conditions (30^0 C-35^0C) in muslin cloth bag. Three sets of 500 seeds each were equilibrated over different saturated salts in airtight desiccators of uniform size to control the relative humidity. The different

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levels of relative humidities were established using Vertucci and Roos (1993) method of specific saturated salt solutions while, the temperature was maintained in an incubator. Under the present investigation, four salts *viz.,* sodium chloride (75%), magnesium chloride (33%), lithium chloride (11%) and zinc chloride (5.5%) were used to equilibrate the seeds to different moisture levels. They were stored at three different temperatures *i e.* 5^{0}C, 20^0 C and ambient (30^{0}C-35^{0}C) temperature storage temperatures and relative humidity were monitored with the help of thermometer and hygrometer, respectively. The seed viability pattern under different conditions was monitored at regular intervals of 30days for four months to assess the changes in various physiological and biochemical characteristics of seeds.

Seed germination test :

Three replications of 25 seeds each were kept for germination in Petri plates using ISTA (1993) method. The emergence of both radical and plumule were recorded after 8th day of placing the seeds.

Electrical conductivity of seed leachates :

The electrical conductivity was measured by weighing three replicates of 20 seeds each and soaking them in 50 ml of deionized water at 25^{0}C for 17 h and conductivity was measured for resultant leachate water using conductivity meter (Control Dynamics, India).

Total soluble proteins :

Seeds (0.5 g) were extracted in 0.01M phosphate buffer (pH 7), precipitated in 10 per cent TCA (Trichloro-acetic acid) re-dissolved in 0.1N NAOH and estimated by Lowry's method (Lowry *et al.,* 1951). The extract was centrifuged at 1,500rpm for 10minutes. 0.5 ml of the extract was taken and to this 5 ml of reagent was added and kept at room temperature for 15 minutes. Then 0.5 ml of Folin- Ciocalteu reagent was added. From this aliquot, soluble protein content was determined using spectrophotometer at 660nm using the standard graph of bovine serum albumin (BSA) and the results were expressed in mg/ g of fresh weight.

Statistical analysis :

Analysis of variance: The data from the laboratory experiment recorded as percentage were transformed to the respective angular (arc sine) values before subjecting them to statistical scrutiny. The data were analyzed statistically by adopting factorial CRD technique (Panse and Sukatme, 1985). Correlation coefficient was also calculated. Differences among means were tested for significance using least significant difference tests (LSD).

RESULTS AND DISCUSSION

The results are summarized below according to objectives of the study:

Seed germination :

The seeds were equilibrated to 12.9 to 11.4 per cent moisture content at 75 per cent relative humidity(RH), 8.5-7.8 per cent moisture content at 33 per cent RH, 4.9-4.1 per cent moisture content at 11 per cent RH and 4.0-3.4 per cent moisture content at 5.5 per cent RH.Amongst the various treatments, 33 per cent RH and 20°C showed significantly higher value for germination (78%) after 120days of storage.Whereas, 5.5 per cent RH and 5^{0}C recorded lower values for germination (17%). Seed germination generally decreased with storage. Germination was maintained in seeds stored at 33 per cent RH throughout the storage period (Fig.1a).

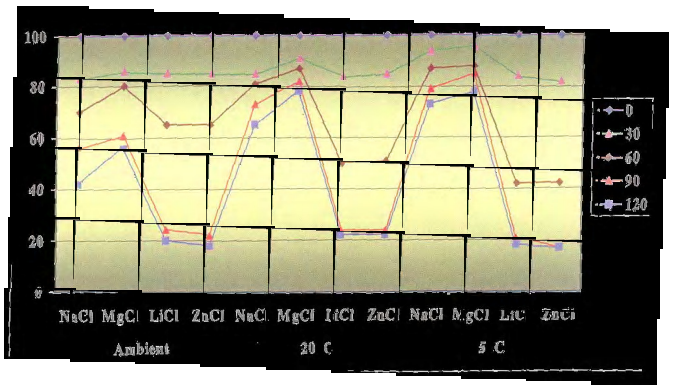

Fig.1a: Effect of storage conditions on seed germination(%) in *Cassia angustifolia*

Vigour index :

Significant reduction in vigour index was observed with storage period in all the conditions. The highest value of vigour index (921.07) was observed at 20^{0}C and 33 per cent RH. Storage under high RH of 75 per cent resulted in a significant fall in the vigour index after 60 days of storage. Seeds stored at all the three temperature showed similar response. Decline in vigour index was observed at all temperature and relative humidity levels when compared to control (Fig. 1b).

Fig. 1b : Effect of storage conditions on vigour index in *Cassia angustifolia*

Total soluble proteins :

The change in the level of proteins during storage were non significant both qualitatively and quantitatively.The amount of soluble protein was found to be 218mg/g fresh weight in fresh seeds, which significantly decreased under storage. At 5.5 per cent and 11 per cent RH and 5^{0}C there was less change in the level of protein whereas, a drastic reduction in the level of proteins was observed under ambient temperature. In general, seeds at lowest humidity (5.5%) maintained the protein levels up to 60 days of storage with insignificant decline in the protein values.

Lipid peroxidation :

Lipid peroxidation values were the least in healthy and 100 per cent viable seeds and showed an increasing trend with seed deterioration in storage (Fig.1c). A significant increase of 10-15 times in the level of lipid peroxidation was observed after 120 days of storage. At 33 per cent RH also the lipid peroxidation values increased through values were recorded under ambient temperature. Similar trend was observed at 5.5 per cent RH where the increase in the value of lipid peroxidation was 2-2.5 times with maximum increase in the seeds stored under ambient conditions, correlating well with the lower/decreased seed viability.

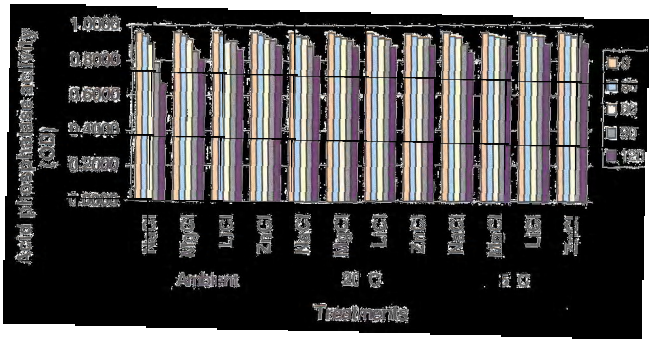

Fig.1c: **Effect of storage conditions on acid phosphotase activity in *Cassia angustifolia***

Enzyme activities :

Activities of both the enzymes dehydrogenase and acid phosphates declined under storage with deterioration of seed. In fresh seeds activity of enzyme dehydrogenase (◆ OD /g fresh weight /ml) was found to be 0.76. Similar activity (0.70 and 0.69) was maintained at 5^{0}C and 20^{0}C under 11 per cent RH and 5.5 per cent RH up to 120days of storage (Fig. 1d). At ambient temperature, the activity was lowered after two months of storage, which was 0.44 and further decreased to 0.31 after 4 month of storage. At higher RH of 75 per cent the activity reduced significantly. In fresh seeds, activity of the enzyme acid phosphates (OD/g fresh weight/ml) was recorded as 0.956 (Fig. 1e). This activity (0.897) was maintained well at 5^{0}C of both 11per cent RH and 5.5 per cent RH.

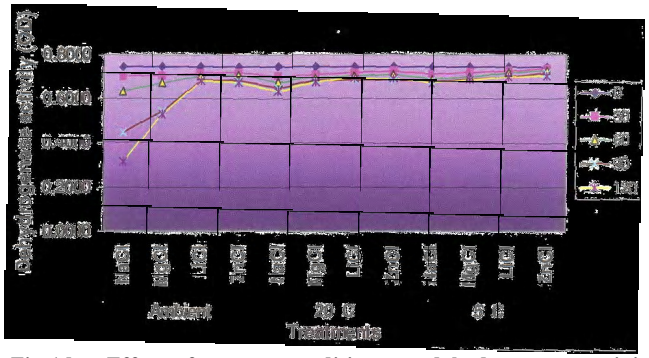

Fig.1d: **Effect of storage conditions on dehydrogenase activity in *Cassia angustifolia***

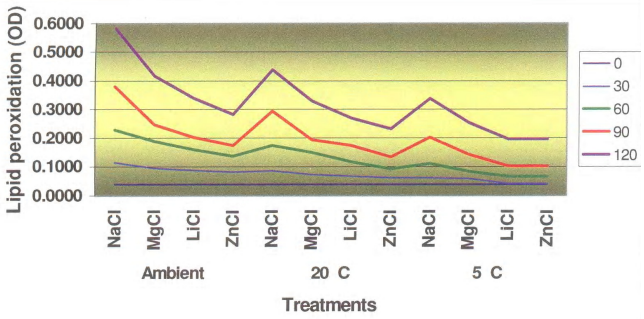

Fig.1e: **3Effect of storage conditions on lipid peroxidation in *Cassia angustifolia***

Protein profile :

The protein profile of the *Cassia angustifolia* seeds under different temperature and RH showed a changed banding pattern with increase in storage period. A band having low mobility (high molecular weight protein) with Rm value of 0.134 which was present in control disappeared after 60 days of storage at ambient temperature and at 75 per cent RH. Numbers of bands were maximum in control (7) while, minimum of 5 bands were observed at ambient temperature and 75 per cent RH (Table 1). A prominent band (Rm0.503), which was present in the control seed, disappeared after storage. There was no change in the number of protein bands in 5.5 per cent RH per cent and 5^{0}C and it could be due to synthesis of certain proteins in response to shock (high temperature and humidity) or due to shifting of seed to dormancy.

Under the present investigation, no adverse effect of desiccation and chilling was observed on the viability of senna seeds as indicated by 96 per cent viability even at a low level of moisture and low temperature conditions. Further it was observed that if the material is dried to water equilibrium, the

Table 1: SDS-PAGE protein profile of *Cassia angustifolia* seeds in different storage conditions

Band No.	RM value	Control	$ZnCl_2$ (5.5%)						LiCl (11%)					
			5^0C		20^0C		Ambient		5^0C		20^0C		Ambient	
			60	120	60	120	60	120	60	120	60	120	60	120
1.	0.049	+	+	+	+	+	+	+	+	+	+	+	+	+
2.	0.134	+	+	+	+	+	+	+	+	+	+	+	+	+
3.	0.063	++	++	++	++	++	++	++	++	++	++	++	++	++
4.	0.028	+	+	+	+	+	+	+	+	+	+	+	+	+
5.	0.056	+	+	+	+	+	+	+	+	+	+	+	+	+
6.	0.503	+	+	+	+	+	+	+	+	+	+	+	+	+
7.	0.140	-	-	-	-	-	-	-	-	-	-	-	-	-
8.	0.366	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Table 1 : Contd……

Band No.	RM value	Control	$Mg Cl_2$ (33%)						NaCl (75%)					
			5^0C		20^0C		Ambient		5^0C		20^0C		Ambient	
			60	120	60	120	60	120	60	120	60	120	60	120
1.	0.049	+	+	+	+	+	+	+	+	+	+	+	+	+
2.	0.134	+	+	+	+	+	+	+	+	+	+	+		
3.	0.063	++	++	++	++	++	++	++	++	++	++	++	++	++
4.	0.028	+	+	+	+	+	+	+	+	+	+	+	+	+
5.	0.056	+	+	+	+	+	+	+	+	+	+	+	+	+
6.	0.503	+	+	+	+	+	+	+	+	+	+	+	+	+
7.	0.140	-	-	-	-	-	-	-	-	-	-	+	+	-
8.	0.366	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Intensity of bands: - absent, +light, ++dark, +++very dark, DAS=Days after storage

loss of viability depends on the time (how long) a material was at that specific water content. Similar results were observed by Chandel *et al.* (1995).

Seed deterioration leading to loss of germination progressed very rapidly at higher humidity and temperature (Harrington, 1973a ; Bass *et al.,* 1963). In the present study, under higher RH(75%) over NaCl, the survival rates were significantly lower compared to the other two conditions of 11per cent and 5.5 per cent RH at all the temperatures. The seeds could retain the viability over 85 per cent only for a short period of one month, indicating that storage of senna seeds is difficult even for a short period in the tropical regions with high temperature and high humidity.

The decrease in vigour index was found to be directly proportional to the seed viability. Loss in seeding vigour is reported to proceed with loss of seed viability in a number of crops (Harrington, 1972 ; Dey and Basu, 1982; Yadav *et al*., 1987; Dharamalingam and Basu,1990). In the present study, decline in seedling vigour preceded with low speed of germination which is in conformity with the earlier results of Raghuveer Rao (1988). Similarly ageing induced loss of vigour was reported by Dey and Mukherjee(1988) in artificially aged mustard seeds. Shanmugavel *et al.* (1995) observed similar reduction in physiological attributes in aged soybean seeds.

The stored senna seeds exhibited a declining trend in the quantity of proteins with time. The decrease in the level of protein with increasing storage time could be due to the denaturation of proteins during storage. Seeds stored under various conditions showed deteriorative changes under high and low humidity conditions after120 days of storage. Protein profile in the present study showed that a higher molecular weight protein broken down in to smaller polypeptides when the seed was exposed to various storage conditions. The appearance of additional band with the Rm value 0.140 could be due to synthesis of certain proteins in the response to the shock (high temperature and high humidity) caused during storage or shifting of seed from maturity to dormancy phase. Similar results were reported in cotton (Anuradha *et al.,* 2003).

Membranes are the most important sites of a seed which appears to be adversely affected by seed deterioration/ ageing (Ching and Schoolcraft, 1968). Derivative changes in cellular membranes are some early of seed ageing.

Both the enzymes dehydrogenase and acid phosphatase activity decreased with storage period. This decrease is more prominent under high temperature and RH conditions (Fig. 1d and 1e). These results are similar with the earlier reports of Chandel *et al.* (1995). These results support the hypothesis that the level of membrane bound enzyme decreases during seed melioration (Li and Sun,1999; Chaitanya and Naithani, 1994; Chandel *et al.,*1995)

Increases in malondialdehyde (MDA) throughout the period of ageing in seeds were reported by several workers (Stewart and Bewley, 1980; Sung and Jeng,1994). Under the present study, there was a progressive increase in the lipid peroxidation during storage in senna seeds (Fig.1c). Lipid peroxidation in biological samples is associated with loss of unsaturated bonds in lipids which can cause extensive disturbance to the ordered structure of membranes.

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